Supplemental Table 1: Summary of primary antibodies used with corresponding target protein, manufacturer, species raised in and dilution used

Peptide/protein target	**Name of Antibody**	**Manufacturer, catalog #, and/or name of individual providing the antibody**	**Species raised in; monoclonal or polyclonal**	**Dilution used**
Total IGFBP-1	Anti human IGFBP-1 monoclonal 6303	Medix Biochemica	Mouse; Monoclonal	1:10000
Total IGFBP-1	Anti human IGFBP-1 polyclonal	Dr. Rob C Baxter	Rabbit: Polyclonal	1:10000
Phospho-IGFBP-1 (S101)	Phospho-IGFBP-1 (S101)	YenZym	Rabbit; Polyclonal	1:1000
Phospho-IGFBP-1 (S119)	Phospho-IGFBP-1 (S119)	YenZym	Rabbit; Polyclonal	1:1000
Phospho-IGFBP-1 (S169)	Phospho-IGFBP-1 (S169)	YenZym	Rabbit; Polyclonal	1:1000
β-actin	β-actin (8H10D10) Mouse mAb	Cell signaling, #3700	Mouse; Monoclonal	1:3000
Phospho-4EBP-1 (T70)	Phospho-4E-BP1 (Thr70) Antibody	Cell signaling, #9455	Rabbit; Polyclonal	1:1000
Total 4EBP-1	4EBP-1 Antibody	Cell signaling, #9452	Rabbit; Polyclonal	1:1000
Phospho-Akt (S473)	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	Cell signaling, #4060	Rabbit; Monoclonal	1:1000
Total Akt	Akt Antibody	Cell signaling, #9272	Rabbit; Polyclonal	1:1000
Phospho-IGF-1Rβ (Y1135)	Phospho-IGF-1 Receptor β (Tyr1135) (DA7A8) Rabbit mAb	Cell signaling, #3918	Rabbit; Monoclonal	1:1000
Total IGF-1Rβ	IGF-1Rβ (C-20)	Santa Cruz Biotechnology, sc-713	Rabbit; Polyclonal	1:1000
Raptor	Raptor (24C12) Rabbit mAb	Cell signaling, #2280	Rabbit; Monoclonal	1:1000
Rictor	Rictor (53A2) Rabbit mAb	Cell signaling, #2114	Rabbit; Monoclonal	1:1000
TSC2	Tuberin/TSC2 (D93F12) Rabbit mAb	Cell signalling, #4308	Rabbit; Monoclonal	1:1000
DEPTOR	DEPTOR/DEPDC6 (D9F5) Rabbit mAb	Cell signalling, #11816	Rabbit; Monoclonal	1:1000

Supplemental Table 2. Dataset for MRM transitions used to detect IGFBP-1 phosphorylated peptides

Precursor ions	Transitions	Peptide Sequence	Collision Energy
765.92	1346.72	sequence1.ALPGEQQPLHALTR.+2y12.light	36
765.92	807.48	sequence1.ALPGEQQPLHALTR.+2y7.light	36
765.92	673.86	sequence1.ALPGEQQPLHALTR.+2y12+2.light	36
765.92	404.25	sequence1.ALPGEQQPLHALTR.+2y7+2.light	36
765.92	724.36	sequence1.ALPGEQQPLHALTR.+2b7.light	36
765.92	1071.56	sequence1.ALPGEQQPLHALTR.+2b10.light	36
773.32	575.26	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y5.light	40
773.32	261.16	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y2.light	40
773.32	944.88	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y15+2.light	40
773.32	861.38	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y14+2.light	40
773.32	426.21	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y7+2.light	40
773.32	574.59	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y14+3.light	40
773.32	376.20	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y9+3.light	40
773.32	230.12	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y6+3.light	40
773.32	200.10	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b2.light	40
773.32	329.15	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b3.light	40
773.32	215.60	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b4+2.light	40
773.32	734.28	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b12+2.light	40
773.32	1086.42	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b18+2.light	40
773.32	199.74	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b5+3.light	40
773.32	218.74	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b6+3.light	40
773.32	342.47	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b9+3.light	40
773.32	489.85	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b12+3.light	40
773.32	652.27	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b16+3.light	40
746.66	575.26	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y5.light	38
746.66	261.16	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y2.light	38
746.66	904.90	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y15+2.light	38
746.66	861.38	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y14+2.light	38
746.66	426.21	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y7+2.light	38
746.66	574.59	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y14+3.light	38
746.66	376.20	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y9+3.light	38
746.66	230.12	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y6+3.light	38
746.66	200.10	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b2.light	38
746.66	329.15	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b3.light	38
746.66	215.60	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b4+2.light	38
746.66	694.29	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b12+2.light	38
746.66	1046.44	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b18+2.light	38
746.66	173.08	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b5+3.light	38
746.66	192.09	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b6+3.light	38
746.66	315.81	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b9+3.light	38
746.66	463.20	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b12+3.light	38
746.66	625.61	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b16+3.light	38
746.66	575.26	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y5.light	38
746.66	261.16	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y2.light	38
746.66	904.90	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y15+2.light	38
746.66	821.40	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y14+2.light	38
746.66	426.21	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y7+2.light	38
746.66	547.94	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y14+3.light	38

746.66	376.20	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y9+3.light	38
Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
746.66	230.12	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y6+3.light	38
746.66	200.10	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b2.light	38
746.66	329.15	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b3.light	38
746.66	215.60	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b4+2.light	38
746.66	694.29	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b12+2.light	38
746.66	1046.44	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b18+2.light	38
746.66	199.74	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b5+3.light	38
746.66	218.74	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b6+3.light	38
746.66	342.47	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b9+3.light	38
746.66	463.20	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b12+3.light	38
746.66	625.61	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b16+3.light	38
720.01	575.26	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y5.light	37
720.01	261.16	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y2.light	37
720.01	864.92	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y15+2.light	37
720.01	821.40	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y14+2.light	37
720.01	426.21	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y7+2.light	37
720.01	547.94	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y14+3.light	37
720.01	376.20	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y9+3.light	37
720.01	230.12	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y6+3.light	37
720.01	200.10	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b2.light	37
720.01	329.15	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b3.light	37
720.01	215.60	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b4+2.light	37
720.01	654.31	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b12+2.light	37
720.01	1006.46	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b18+2.light	37
720.01	173.08	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b5+3.light	37
720.01	192.09	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b6+3.light	37
720.01	315.81	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b9+3.light	37
720.01	436.54	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b12+3.light	37
720.01	598.96	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b16+3.light	37
976.40	1076.54	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y9.light	51
976.40	975.49	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y8.light	51
976.40	503.27	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y4.light	51
976.40	245.19	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y2.light	51
976.40	1292.02	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y23+2.light	51
976.40	1103.93	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y19+2.light	51
976.40	1003.89	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y17+2.light	51
976.40	931.87	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y15+2.light	51
976.40	883.34	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y14+2.light	51
976.40	735.32	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y12+2.light	51
976.40	488.25	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y8+2.light	51
976.40	621.58	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y15+3.light	51
976.40	458.20	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y11+3.light	51

Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
976.40	978.43	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b11.light	51
976.40	1458.55	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b15.light	51
976.40	173.07	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b4+2.light	51
976.40	1097.91	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b21+2.light	51
976.40	388.18	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b13+3.light	51
976.40	562.22	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b17+3.light	51
949.74	1076.54	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y9.light	49
949.74	975.49	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y8.light	49
949.74	503.27	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y4.light	49
949.74	245.19	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y2.light	49
949.74	1252.04	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y23+2.light	49
949.74	1063.95	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y19+2.light	49
949.74	963.91	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y17+2.light	49
949.74	891.88	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y15+2.light	49
949.74	843.36	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y14+2.light	49
949.74	735.32	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y12+2.light	49
949.74	488.25	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y8+2.light	49
949.74	594.92	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y15+3.light	49
949.74	458.20	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y11+3.light	49
949.74	978.43	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b11.light	49
949.74	1378.59	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b15.light	49
949.74	173.07	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b4+2.light	49
949.74	1057.93	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b21+2.light	49
949.74	388.18	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b13+3.light	49
949.74	535.57	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b17+3.light	49
923.09	1076.54	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y9.light	48
923.09	975.49	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y8.light	48
923.09	503.27	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y4.light	48
923.09	245.19	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y2.light	48
923.09	1212.06	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y23+2.light	48
923.09	1023.97	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y19+2.light	48
923.09	923.93	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y17+2.light	48
923.09	851.90	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y15+2.light	48
923.09	803.37	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y14+2.light	48
923.09	695.34	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y12+2.light	48
923.09	488.25	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y8+2.light	48
923.09	568.27	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y15+3.light	48
923.09	431.54	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y11+3.light	48
923.09	978.43	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b11.light	48
923.09	1378.59	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b15.light	48
923.09	173.07	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b4+2.light	48
923.09	1017.95	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b21+2.light	48
923.09	388.18	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b13+3.light	48
923.09	535.57	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b17+3.light	48

Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
685.26	541.15	sequence1.DNFHLMAPS[Pho]EE.+2y4.light	34
685.26	428.66	sequence1.DNFHLMAPS[Pho]EE.+2y7+2.light	34
685.26	372.12	sequence1.DNFHLMAPS[Pho]EE.+2y6+2.light	34
685.26	306.60	sequence1.DNFHLMAPS[Pho]EE.+2y5+2.light	34
685.26	230.08	sequence1.DNFHLMAPS[Pho]EE.+2b2.light	34
685.26	377.15	sequence1.DNFHLMAPS[Pho]EE.+2b3.light	34
685.26	829.37	sequence1.DNFHLMAPS[Pho]EE.+2b7.light	34
685.26	463.71	sequence1.DNFHLMAPS[Pho]EE.+2b8+2.light	34
685.26	547.21	sequence1.DNFHLMAPS[Pho]EE.+2b9+2.light	34
645.28	461.19	sequence1.DNFHLMAPSEE.+2y4.light	32
645.28	388.68	sequence1.DNFHLMAPSEE.+2y7+2.light	32
645.28	332.14	sequence1.DNFHLMAPSEE.+2y6+2.light	32
645.28	266.62	sequence1.DNFHLMAPSEE.+2y5+2.light	32
645.28	230.08	sequence1.DNFHLMAPSEE.+2b2.light	32
645.28	377.15	sequence1.DNFHLMAPSEE.+2b3.light	32
645.28	829.37	sequence1.DNFHLMAPSEE.+2b7.light	32
645.28	463.71	sequence1.DNFHLMAPSEE.+2b8+2.light	32
645.28	507.23	sequence1.DNFHLMAPSEE.+2b9+2.light	32

Table S3. IGFBP-1 phosphopeptide peak intensity using MRM MS analysis of cell media from rapamycin and hypoxia treated HepG2 cells

Precursor ion	PeptideSequence	Peak Intensity (Control)	Peak Intensity (Rapa)	Peak Intensity (Hypoxia)	Peak Intensity (Rapa + Hypoxia)
765.92	ALPGEQQPLHALTR	12578	23741	5416	4880
773.32	AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK	8470	24962	24273	6555
746.66	AQETS[Pho]GEEISKFYLPNC[CAM]NK	7729	33732	7819	10280
720.01	AQETSGEEISKFYLPNC[CAM]NK	8123	13649	7463	5193
976.40	DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL	19386	9227	13799	11543
949.74	DASAPHAAEAGSPESPES[Pho]TEITEEELL	16342	7260	11088	17250
923.09	DASAPHAAEAGSPESPESTEITEEELL	6458	1513	3245	2875
685.26	DNFHLMAPS[Pho]EE	19386	9227	13799	11543
645.28	DNFHLMAPSEE	8764	1624	1589	1657

Table S4. IGFBP-1 phosphopeptide peak intensity using MRM MS analysis of cell media from HepG2 cells treated for mTORC1 + C2 activation by DEPTOR silencing

Precursor ion	PeptideSequence	Peak Intensity (Scrambled siRNA)	Peak Intensity (DEPTOR siRNA)	Peak Intensity (DEPTOR siRNA + Hypoxia)
773.32	AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK	8215	704	578
746.66	AQETS[Pho]GEEISKFYLPNC[CAM]NK	740	211	234
746.66	AQETSGEEIS[Pho]KFYLPNC[CAM]NK	881	193	251
720.01	AQETSGEEISKFYLPNC[CAM]NK	9526	7548	4898

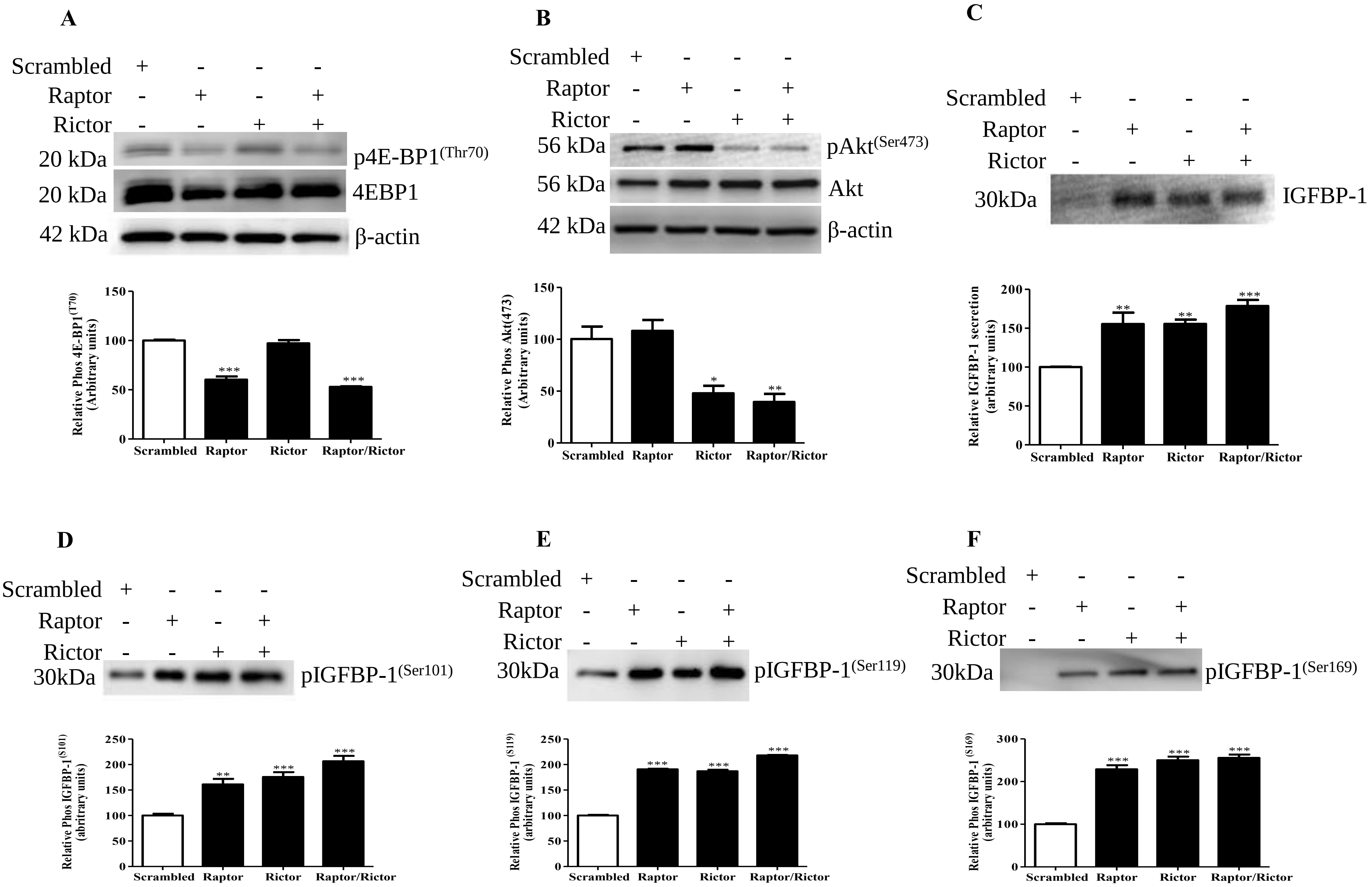

A
Scrambled + - - -
Raptor - + - +
Rictor - - + +
20 kDa p4E-BP1(Thr70)
20 kDa 4EBP1
42 kDa β-actin
Relative Phos 4E-BP1(T70) (Arbitrary units)
150
100
50
0
***
***
Scrambled Raptor Rictor Raptor/Rictor
B
Scrambled + - - -
Raptor - + - +
Rictor - - + +
56 kDa pAkt(Ser473)
56 kDa Akt
42 kDa β-actin
Relative Phos Akt(473) (Arbitrary units)
150
100
50
0
*
**
Scrambled Raptor Rictor Raptor/Rictor
C
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa IGFBP-1
Relative IGFBP-1 secretion (arbitrary units)
200
150
100
50
0
**
**
***
Scrambled Raptor Rictor Raptor/Rictor
D
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser101)
Relative Phos IGFBP-1(S101) (arbitrary units)
250
200
150
100
50
0
**
***
***
Scrambled Raptor Rictor Raptor/Rictor
E
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser119)
Relative Phos IGFBP-1(S119) (arbitrary units)
250
200
150
100
50
0
***
***
***
Scrambled Raptor Rictor Raptor/Rictor
F
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser169)
Relative Phos IGFBP-1(S169) (arbitrary units)
300
200
100
0
***
***
***
Scrambled Raptor Rictor Raptor/Rictor

Figure S1 (A-F)

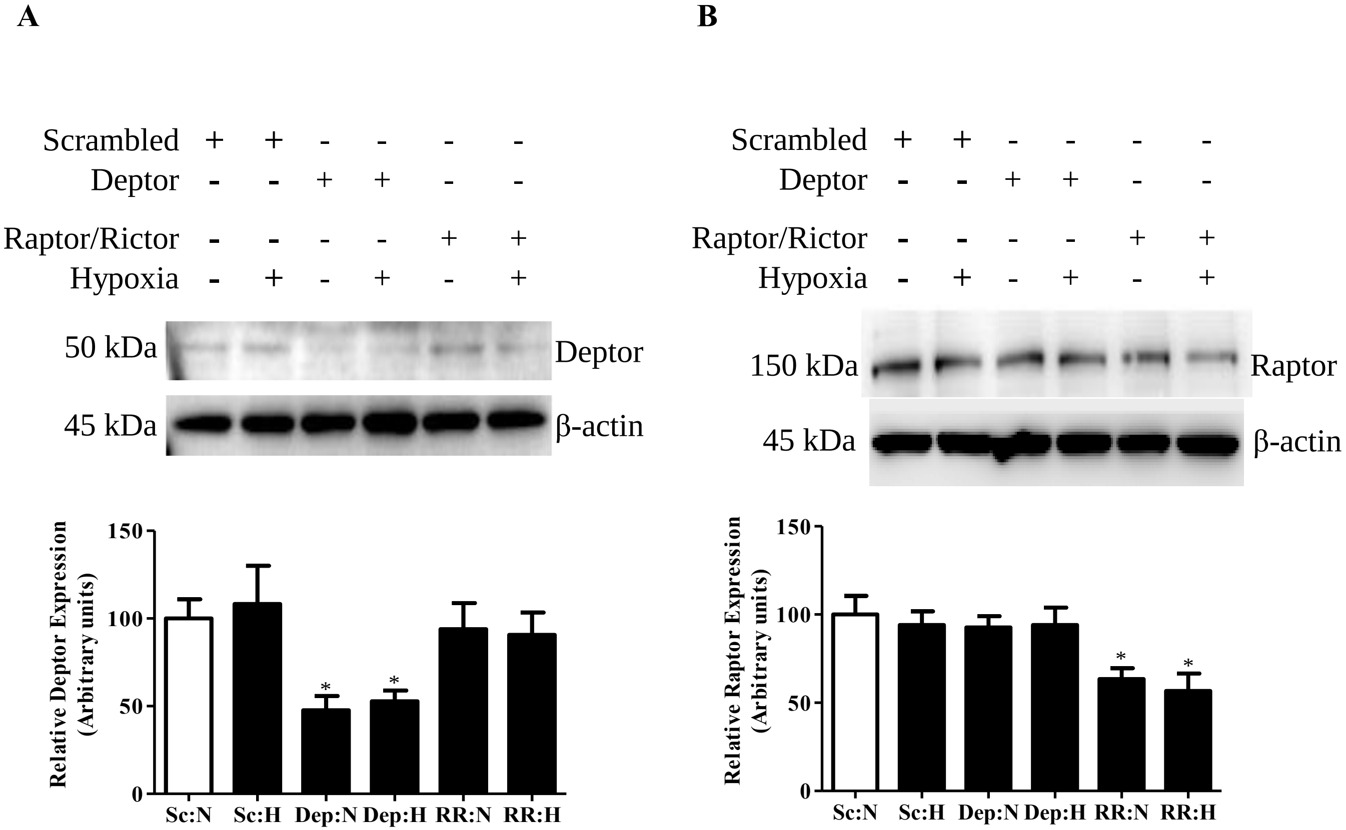

A
Scrambled + + - - - -
Deptor - - + + - -
Raptor/Rictor - - - - + +
Hypoxia - + - + - +
50 kDa
Deptor
45 kDa
β-actin
Relative Deptor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
Dep:N
Dep:H
RR:N
RR:H
B
Scrambled + + - - - -
Deptor - - + + - -
Raptor/Rictor - - - - + +
Hypoxia - + - + - +
150 kDa
Raptor
45 kDa
β-actin
Relative Raptor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
Dep:N
Dep:H
RR:N
RR:H

Figure S2 (A-B)

C

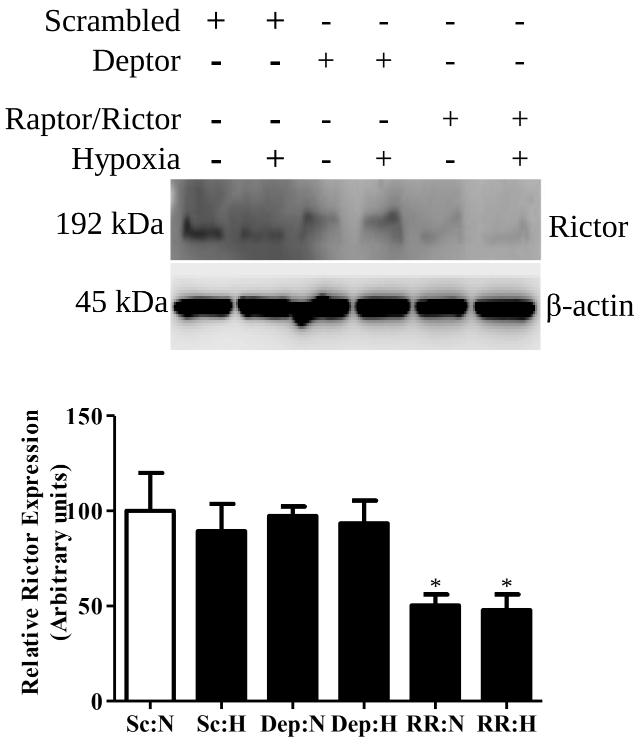

Scrambled + + - - - -
Deptor - - + + - -
Raptor/Rictor - - - - + +
Hypoxia - + - + - +
192 kDa
Rictor
45 kDa
β-actin
Relative Rictor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
Dep:N
Dep:H
RR:N
RR:H

Figure S2 (C)

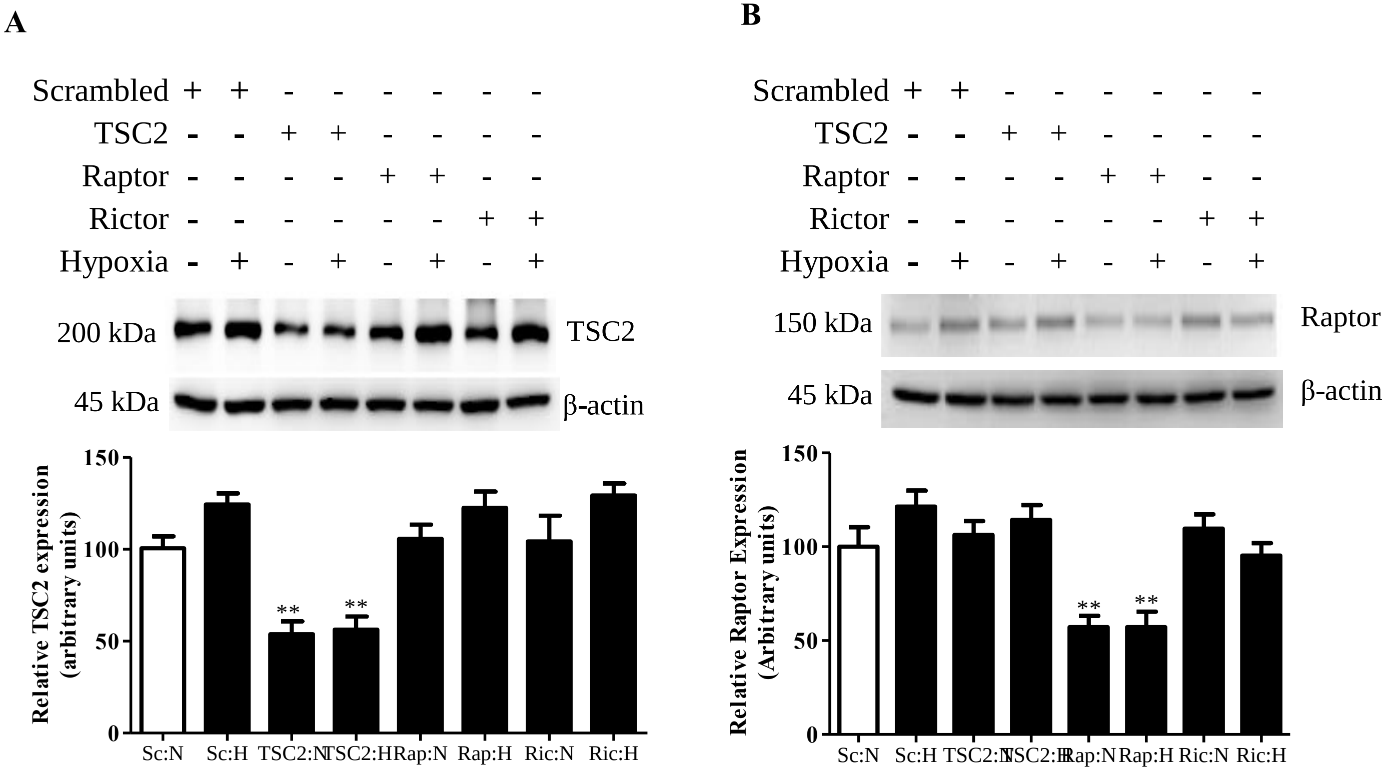

A
Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
200 kDa
TSC2
45 kDa
β-actin
Relative TSC2 expression (arbitrary units)
150
100
50
0
**
**
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H
B
Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
150 kDa
Raptor
45 kDa
β-actin
Relative Raptor Expression (Arbitrary units)
150
100
50
0
**
**
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H

Figure S3 (A-B)

C

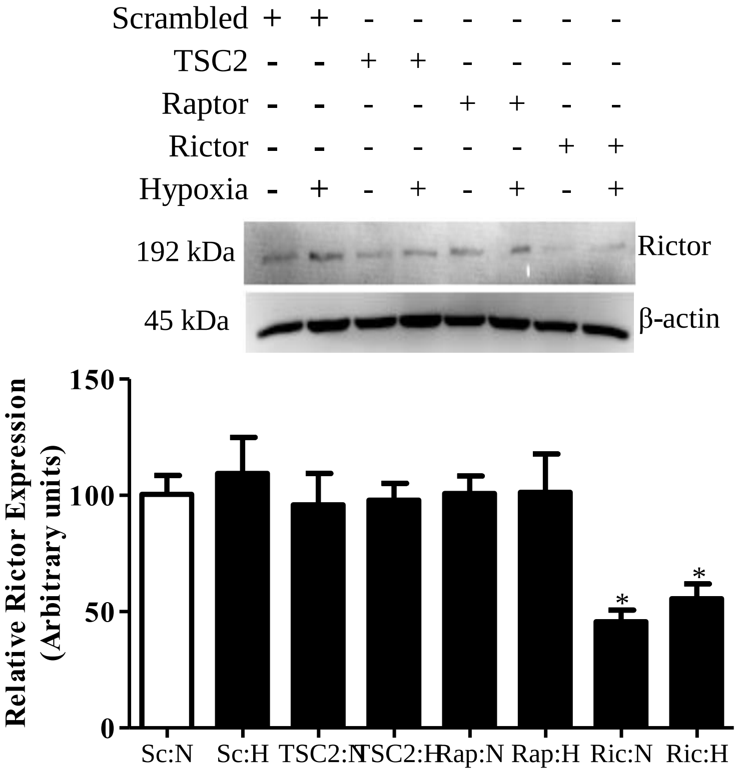

Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
192 kDa
Rictor
45 kDa
β-actin
Relative Rictor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H

Figure S3 (C)

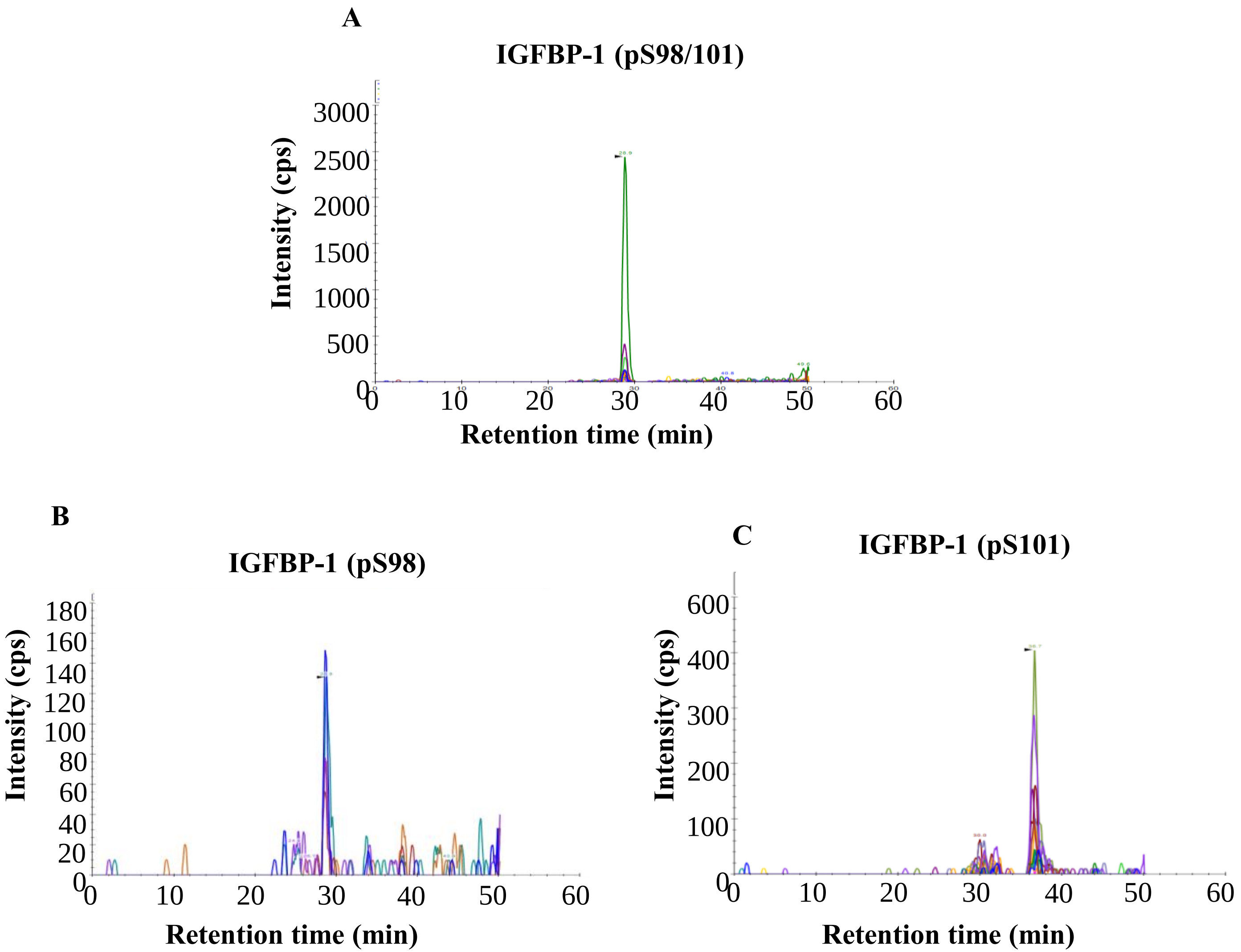

A
IGFBP-1 (pS98/101)
Intensity (cps)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Retention time (min)
B
IGFBP-1 (pS98)
Intensity (cps)
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Retention time (min)
C
IGFBP-1 (pS101)
Intensity (cps)
600
400
300
200
100
0
0
10
20
30
40
50
60
Retention time (min)

Figure S4 (A-C)

D

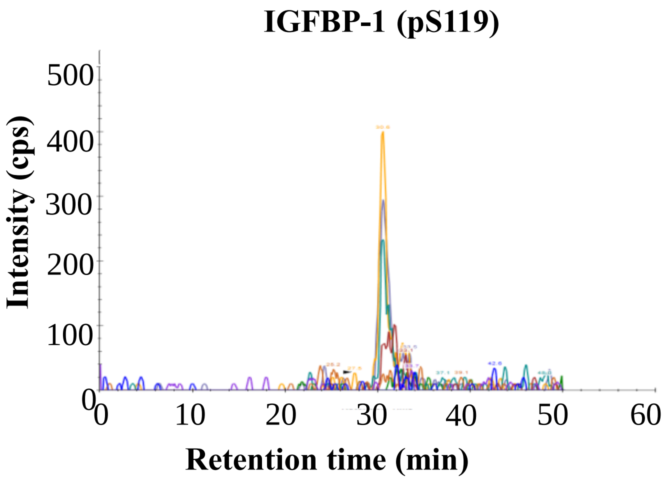
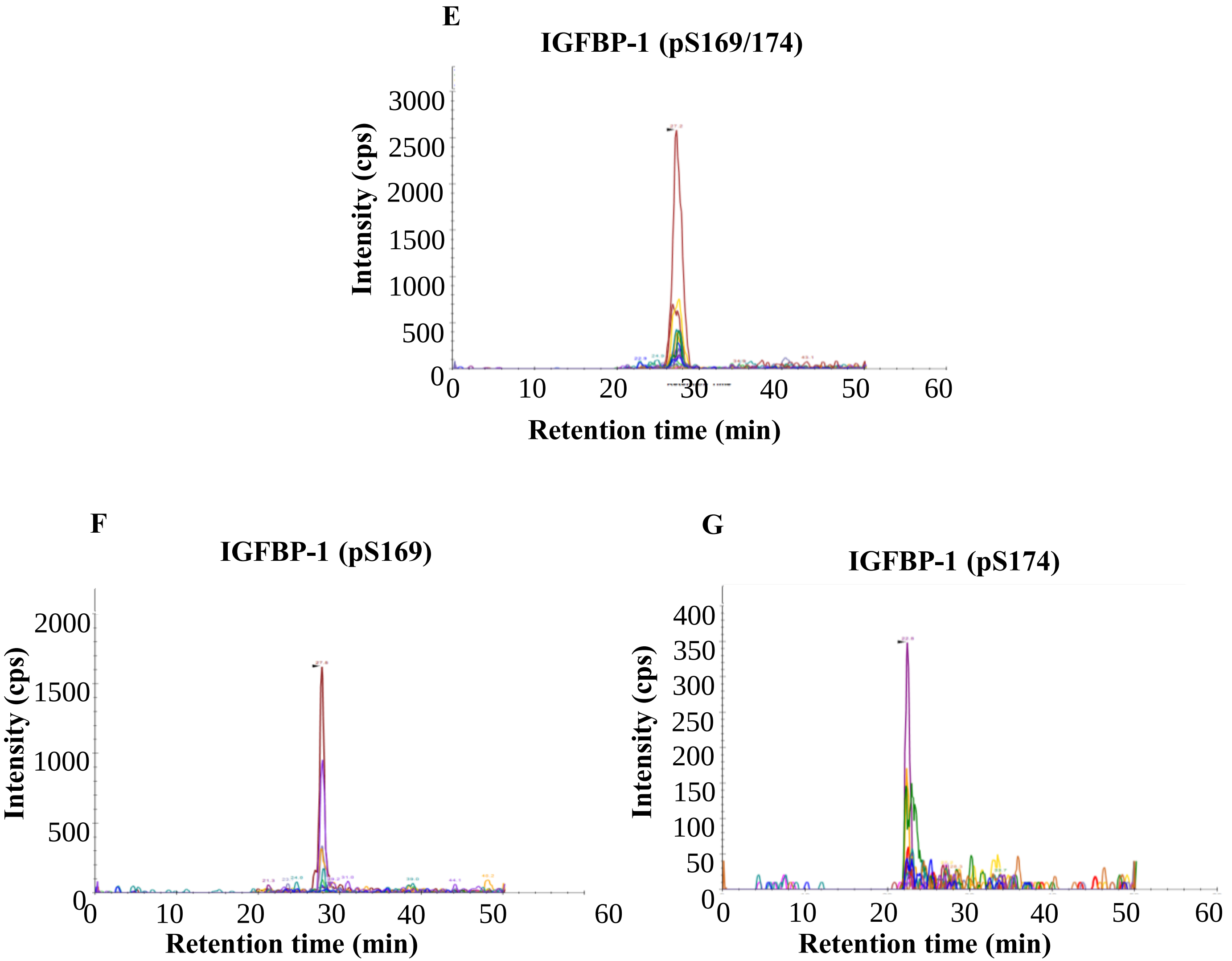

IGFBP-1 (pS119)
Intensity (cps)
500
400
300
200
100
0
0
10
20
30
40
50
60
Retention time (min)

Figure S4 (D)



E

IGFBP-1 (pS169/174)

F

IGFBP-1 (pS169)

G

IGFBP-1 (pS174)

Figure S4 (E-G)

H **Standard IGFBP-1 internal peptide**

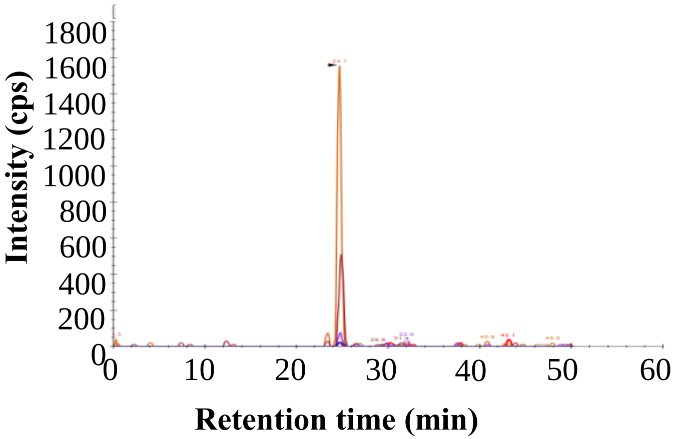
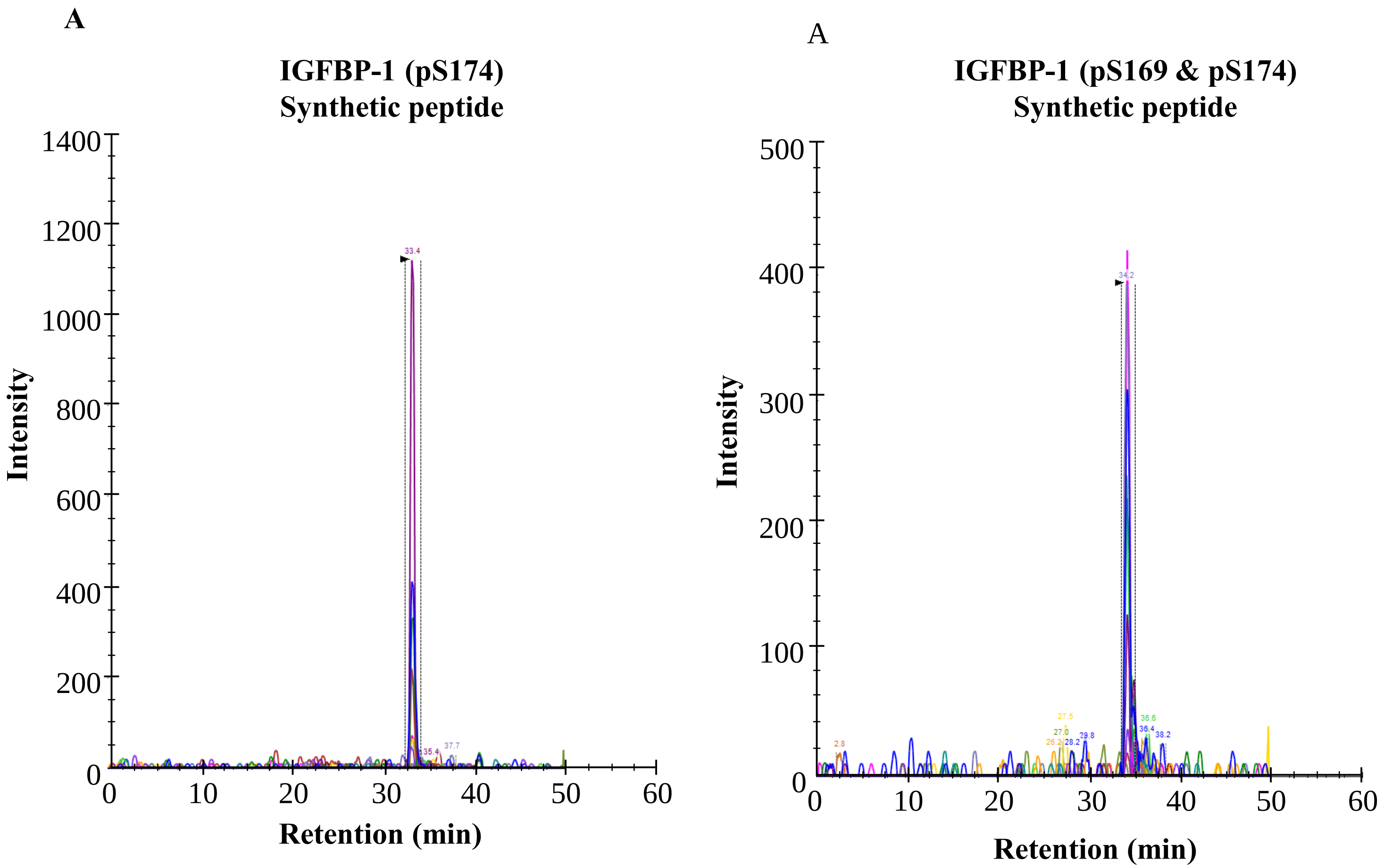


Figure S4 (H)



A

IGFBP-1 (pS174)
Synthetic peptide

A

IGFBP-1 (pS169 & pS174)
Synthetic peptide

Figure S5 (A-B)

Supplemental Figure 1. The effect of raptor and rictor silencing on IGFBP-1 secretion and phosphorylation

Representative western blot of A. mTORC1 readout 4E-BP1 (Thr70) and B. mTORC2 readout Akt (Ser473) phosphorylation in HepG2 cell lysates. Raptor and raptor+rictor silencing inhibited 4E-BP1 phosphorylation while rictor and raptor+rictor silencing both inhibited Akt phosphorylation to similar degrees. Western blot using equal aliquots of cell media of scrambled, raptor, rictor, and combined raptor+rictor siRNA treated HepG2 cells. Raptor, rictor, and combined raptor+rictor siRNA all significantly increased. **C.** IGFBP-1 secretion and **D-F.** IGFBP-1 phosphorylation at Ser101, Ser119 and Ser169, suggesting that inhibition of either complex is sufficient to induce IGFBP-1 secretion and phosphorylation. Values are displayed as mean ± SEM. *$p < 0.05$, **$p = 0.001$-0.05, ***$p < 0.0001$;

Supplemental Figure 2. Validation of DEPTOR, raptor, and rictor siRNA on respective protein expression levels under hypoxia

Representative western blots of equal concentration of cell lysate protein from HepG2 cells transfected with scramble (normoxia), scramble (hypoxia), DEPTOR (normoxia), DEPTOR (hypoxia), raptor+rictor (normoxia) and raptor+rictor (hypoxia) siRNA. siRNA silencing of **A.** DEPTOR, B. Raptor and C. Rictor. DEPTOR significantly decreased total DEPTOR protein expression, regardless of hypoxic status. DEPTOR silencing did not affect either raptor or rictor protein expression. Raptor siRNA silencing decreased only raptor protein expression regardless of hypoxic status. Similarly rictor siRNA silencing specifically decreased rictor protein expression regardless of hypoxic status. Values are displayed as mean± SEM. *$p < 0.05$, **$p =$

0.001-0.05, ***$p <$ 0.0001. versus control; One-way analysis of variance; Dunnet's Multiple Comparison Test.

Supplemental Figure 3. Validation of TSC2, raptor, and rictor siRNA on respective protein expression levels under hypoxia

Representative western blots of equal cell lysate protein from HepG2 cells transfected with scramble (normoxia), scramble (hypoxia), TSC2 (normoxia), TSC2 (hypoxia), raptor (normoxia), raptor (hypoxia), rictor (normoxia) and rictor (hypoxia) siRNA. siRNA silencing of **A**. TSC2, **B**. Raptor and **C**. Rictor. TSC2 significantly decreased total TSC2 protein expression regardless of hypoxic status. Raptor siRNA significantly decreased specifically total raptor protein expression, regardless of hypoxic status. Rictor siRNA significantly decreased only rictor protein expression, regardless of hypoxic status. Values are displayed as mean ± SEM. *$p< 0.05$, **$p=$ 0.001-0.05, ***$p <$ 0.0001. versus control; One-way analysis of variance; Dunnet's Multiple Comparison Test.

Supplemental Figure 4. Representative MRM spectra of identified phosphorylation sites

Immunoprecipitated IGFBP-1 samples or a standard synthetic IGFBP-1 internal peptide was digested by respective enzymes to generate appropriate peptide fragments. MRM transitions were predicted by Skyline and the transitions **A-H** representing the modification sites on IGFBP-1 singly and in combination were chosen to distinguish from other serine residues.

Supplemental Figure 5. MRM spectra of synthetic IGFBP-1 peptides containing the novel Ser174 phosphorylation modification

Synthetic peptides of the digested fragment of IGFBP-1 containing either **A**. pSer174 or **B**. pSer169+pSer174 phosphorylation. Synthetic peptides were used to validate the transitions used to detect the novel pSer174 phosphorylation site within IGFBP-1.

Supplemental Table 1 Summary of primary antibodies used with corresponding target protein, manufacturer, species raised in and dilution used

Peptide/protein target	**Name of Antibody**	**Manufacturer, catalog #, and/or name of individual providing the antibody**	**Species raised in; monoclonal or polyclonal**	**Dilution used**
Total IGFBP-1	Anti human IGFBP-1 monoclonal 6303	Medix Biochemica	Mouse; Monoclonal	1:10000
Total IGFBP-1	Anti human IGFBP-1 polyclonal	Dr. Rob C Baxter	Rabbit: Polyclonal	1:10000
Phospho-IGFBP-1 (S101)	Phospho-IGFBP-1 (S101)	YenZym	Rabbit; Polyclonal	1:1000
Phospho-IGFBP-1 (S119)	Phospho-IGFBP-1 (S119)	YenZym	Rabbit; Polyclonal	1:1000
Phospho-IGFBP-1 (S169)	Phospho-IGFBP-1 (S169)	YenZym	Rabbit; Polyclonal	1:1000
β-actin	β-actin (8H10D10) Mouse mAb	Cell signaling, #3700	Mouse; Monoclonal	1:3000
Phospho-4EBP-1 (T70)	Phospho-4E-BP1 (Thr70) Antibody	Cell signaling, #9455	Rabbit; Polyclonal	1:1000
Total 4EBP-1	4EBP-1 Antibody	Cell signaling, #9452	Rabbit; Polyclonal	1:1000
Phospho-Akt (S473)	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	Cell signaling, #4060	Rabbit; Monoclonal	1:1000
Total Akt	Akt Antibody	Cell signaling, #9272	Rabbit; Polyclonal	1:1000
Phospho-IGF-1Rβ (Y1135)	Phospho-IGF-1 Receptor β (Tyr1135) (DA7A8) Rabbit mAb	Cell signaling, #3918	Rabbit; Monoclonal	1:1000
Total IGF-1Rβ	IGF-1Rβ (C-20)	Santa Cruz Biotechnology, sc-713	Rabbit; Polyclonal	1:1000
Raptor	Raptor (24C12) Rabbit mAb	Cell signaling, #2280	Rabbit; Monoclonal	1:1000
Rictor	Rictor (53A2) Rabbit mAb	Cell signaling, #2114	Rabbit; Monoclonal	1:1000
TSC2	Tuberin/TSC2 (D93F12) Rabbit mAb	Cell signalling, #4308	Rabbit; Monoclonal	1:1000
DEPTOR	DEPTOR/DEPDC6 (D9F5) Rabbit mAb	Cell signalling, #11816	Rabbit; Monoclonal	1:1000

Supplemental Table 2. Dataset for MRM transitions used to detect IGFBP-1 phosphorylated peptides

Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
765.92	1346.72	sequence1.ALPGEQQPLHALTR.+2y12.light	36
765.92	807.48	sequence1.ALPGEQQPLHALTR.+2y7.light	36
765.92	673.86	sequence1.ALPGEQQPLHALTR.+2y12+2.light	36
765.92	404.25	sequence1.ALPGEQQPLHALTR.+2y7+2.light	36
765.92	724.36	sequence1.ALPGEQQPLHALTR.+2b7.light	36
765.92	1071.56	sequence1.ALPGEQQPLHALTR.+2b10.light	36
773.32	575.26	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y5.light	40
773.32	261.16	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y2.light	40
773.32	944.88	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y15+2.light	40
773.32	861.38	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y14+2.light	40
773.32	426.21	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y7+2.light	40
773.32	574.59	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y14+3.light	40
773.32	376.20	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y9+3.light	40
773.32	230.12	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3y6+3.light	40
773.32	200.10	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b2.light	40
773.32	329.15	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b3.light	40
773.32	215.60	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b4+2.light	40
773.32	734.28	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b12+2.light	40
773.32	1086.42	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b18+2.light	40
773.32	199.74	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b5+3.light	40
773.32	218.74	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b6+3.light	40
773.32	342.47	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b9+3.light	40
773.32	489.85	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b12+3.light	40
773.32	652.27	sequence1.AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK.+3b16+3.light	40
746.66	575.26	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y5.light	38
746.66	261.16	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y2.light	38
746.66	904.90	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y15+2.light	38
746.66	861.38	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y14+2.light	38
746.66	426.21	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y7+2.light	38
746.66	574.59	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y14+3.light	38
746.66	376.20	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y9+3.light	38
746.66	230.12	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3y6+3.light	38
746.66	200.10	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b2.light	38
746.66	329.15	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b3.light	38
746.66	215.60	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b4+2.light	38
746.66	694.29	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b12+2.light	38
746.66	1046.44	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b18+2.light	38
746.66	173.08	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b5+3.light	38
746.66	192.09	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b6+3.light	38
746.66	315.81	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b9+3.light	38
746.66	463.20	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b12+3.light	38
746.66	625.61	sequence1.AQETSGEEIS[Pho]KFYLPNC[CAM]NK.+3b16+3.light	38
746.66	575.26	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y5.light	38
746.66	261.16	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y2.light	38
746.66	904.90	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y15+2.light	38
746.66	821.40	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y14+2.light	38
746.66	426.21	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y7+2.light	38
746.66	547.94	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y14+3.light	38

746.66	376.20	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y9+3.light	38
Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
746.66	230.12	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3y6+3.light	38
746.66	200.10	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b2.light	38
746.66	329.15	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b3.light	38
746.66	215.60	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b4+2.light	38
746.66	694.29	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b12+2.light	38
746.66	1046.44	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b18+2.light	38
746.66	199.74	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b5+3.light	38
746.66	218.74	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b6+3.light	38
746.66	342.47	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b9+3.light	38
746.66	463.20	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b12+3.light	38
746.66	625.61	sequence1.AQETS[Pho]GEEISKFYLPNC[CAM]NK.+3b16+3.light	38
720.01	575.26	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y5.light	37
720.01	261.16	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y2.light	37
720.01	864.92	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y15+2.light	37
720.01	821.40	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y14+2.light	37
720.01	426.21	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y7+2.light	37
720.01	547.94	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y14+3.light	37
720.01	376.20	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y9+3.light	37
720.01	230.12	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3y6+3.light	37
720.01	200.10	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b2.light	37
720.01	329.15	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b3.light	37
720.01	215.60	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b4+2.light	37
720.01	654.31	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b12+2.light	37
720.01	1006.46	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b18+2.light	37
720.01	173.08	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b5+3.light	37
720.01	192.09	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b6+3.light	37
720.01	315.81	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b9+3.light	37
720.01	436.54	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b12+3.light	37
720.01	598.96	sequence1.AQETSGEEISKFYLPNC[CAM]NK.+3b16+3.light	37
976.40	1076.54	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y9.light	51
976.40	975.49	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y8.light	51
976.40	503.27	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y4.light	51
976.40	245.19	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y2.light	51
976.40	1292.02	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y23+2.light	51
976.40	1103.93	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y19+2.light	51
976.40	1003.89	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y17+2.light	51
976.40	931.87	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y15+2.light	51
976.40	883.34	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y14+2.light	51
976.40	735.32	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y12+2.light	51
976.40	488.25	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y8+2.light	51
976.40	621.58	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y15+3.light	51
976.40	458.20	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3y11+3.light	51

Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
976.40	978.43	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b11.light	51
976.40	1458.55	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b15.light	51
976.40	173.07	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b4+2.light	51
976.40	1097.91	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b21+2.light	51
976.40	388.18	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b13+3.light	51
976.40	562.22	sequence1.DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL.+3b17+3.light	51
949.74	1076.54	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y9.light	49
949.74	975.49	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y8.light	49
949.74	503.27	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y4.light	49
949.74	245.19	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y2.light	49
949.74	1252.04	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y23+2.light	49
949.74	1063.95	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y19+2.light	49
949.74	963.91	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y17+2.light	49
949.74	891.88	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y15+2.light	49
949.74	843.36	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y14+2.light	49
949.74	735.32	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y12+2.light	49
949.74	488.25	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y8+2.light	49
949.74	594.92	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y15+3.light	49
949.74	458.20	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3y11+3.light	49
949.74	978.43	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b11.light	49
949.74	1378.59	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b15.light	49
949.74	173.07	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b4+2.light	49
949.74	1057.93	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b21+2.light	49
949.74	388.18	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b13+3.light	49
949.74	535.57	sequence1.DASAPHAAEAGSPESPES[Pho]TEITEEELL.+3b17+3.light	49
923.09	1076.54	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y9.light	48
923.09	975.49	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y8.light	48
923.09	503.27	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y4.light	48
923.09	245.19	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y2.light	48
923.09	1212.06	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y23+2.light	48
923.09	1023.97	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y19+2.light	48
923.09	923.93	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y17+2.light	48
923.09	851.90	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y15+2.light	48
923.09	803.37	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y14+2.light	48
923.09	695.34	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y12+2.light	48
923.09	488.25	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y8+2.light	48
923.09	568.27	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y15+3.light	48
923.09	431.54	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3y11+3.light	48
923.09	978.43	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b11.light	48
923.09	1378.59	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b15.light	48
923.09	173.07	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b4+2.light	48
923.09	1017.95	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b21+2.light	48
923.09	388.18	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b13+3.light	48
923.09	535.57	sequence1.DASAPHAAEAGSPESPESTEITEEELL.+3b17+3.light	48

Precursor ions	**Transitions**	**Peptide Sequence**	**Collision Energy**
685.26	541.15	sequence1.DNFHLMAPS[Pho]EE.+2y4.light	34
685.26	428.66	sequence1.DNFHLMAPS[Pho]EE.+2y7+2.light	34
685.26	372.12	sequence1.DNFHLMAPS[Pho]EE.+2y6+2.light	34
685.26	306.60	sequence1.DNFHLMAPS[Pho]EE.+2y5+2.light	34
685.26	230.08	sequence1.DNFHLMAPS[Pho]EE.+2b2.light	34
685.26	377.15	sequence1.DNFHLMAPS[Pho]EE.+2b3.light	34
685.26	829.37	sequence1.DNFHLMAPS[Pho]EE.+2b7.light	34
685.26	463.71	sequence1.DNFHLMAPS[Pho]EE.+2b8+2.light	34
685.26	547.21	sequence1.DNFHLMAPS[Pho]EE.+2b9+2.light	34
645.28	461.19	sequence1.DNFHLMAPSEE.+2y4.light	32
645.28	388.68	sequence1.DNFHLMAPSEE.+2y7+2.light	32
645.28	332.14	sequence1.DNFHLMAPSEE.+2y6+2.light	32
645.28	266.62	sequence1.DNFHLMAPSEE.+2y5+2.light	32
645.28	230.08	sequence1.DNFHLMAPSEE.+2b2.light	32
645.28	377.15	sequence1.DNFHLMAPSEE.+2b3.light	32
645.28	829.37	sequence1.DNFHLMAPSEE.+2b7.light	32
645.28	463.71	sequence1.DNFHLMAPSEE.+2b8+2.light	32
645.28	507.23	sequence1.DNFHLMAPSEE.+2b9+2.light	32

Supplemental Table 3. IGFBP-1 phosphopeptide peak intensity using MRM MS analysis of cell media from rapamycin and hypoxia treated HepG2 cells

Precursor ion	**PeptideSequence**	**Peak Intensity (Control)**	**Peak Intensity (Rapa)**	**Peak Intensity (Hypoxia)**	**Peak Intensity (Rapa + Hypoxia)**
765.92	ALPGEQQPLHALTR	12578	23741	5416	4880
773.32	AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK	8470	24962	24273	6555
746.66	AQETS[Pho]GEEISKFYLPNC[CAM]NK	7729	33732	7819	10280
720.01	AQETSGEEISKFYLPNC[CAM]NK	8123	13649	7463	5193
976.40	DASAPHAAEAGSPES[Pho]PES[Pho]TEITEEELL	19386	9227	13799	11543
949.74	DASAPHAAEAGSPESPES[Pho]TEITEEELL	16342	7260	11088	17250
923.09	DASAPHAAEAGSPESPESTEITEEELL	6458	1513	3245	2875
685.26	DNFHLMAPS[Pho]EE	19386	9227	13799	11543
645.28	DNFHLMAPSEE	8764	1624	1589	1657

Table S4. IGFBP-1 phosphopeptide peak intensity using MRM MS analysis of cell media from HepG2 cells treated for mTORC1 + C2 activation by DEPTOR silencing

Precursor ion	PeptideSequence	Peak Intensity (Scrambled siRNA)	Peak Intensity (DEPTOR siRNA)	Peak Intensity (DEPTOR siRNA + Hypoxia)
773.32	AQETS[Pho]GEEIS[Pho]KFYLPNC[CAM]NK	8215	704	578
746.66	AQETS[Pho]GEEISKFYLPNC[CAM]NK	740	211	234
746.66	AQETSGEEIS[Pho]KFYLPNC[CAM]NK	881	193	251
720.01	AQETSGEEISKFYLPNC[CAM]NK	9526	7548	4898

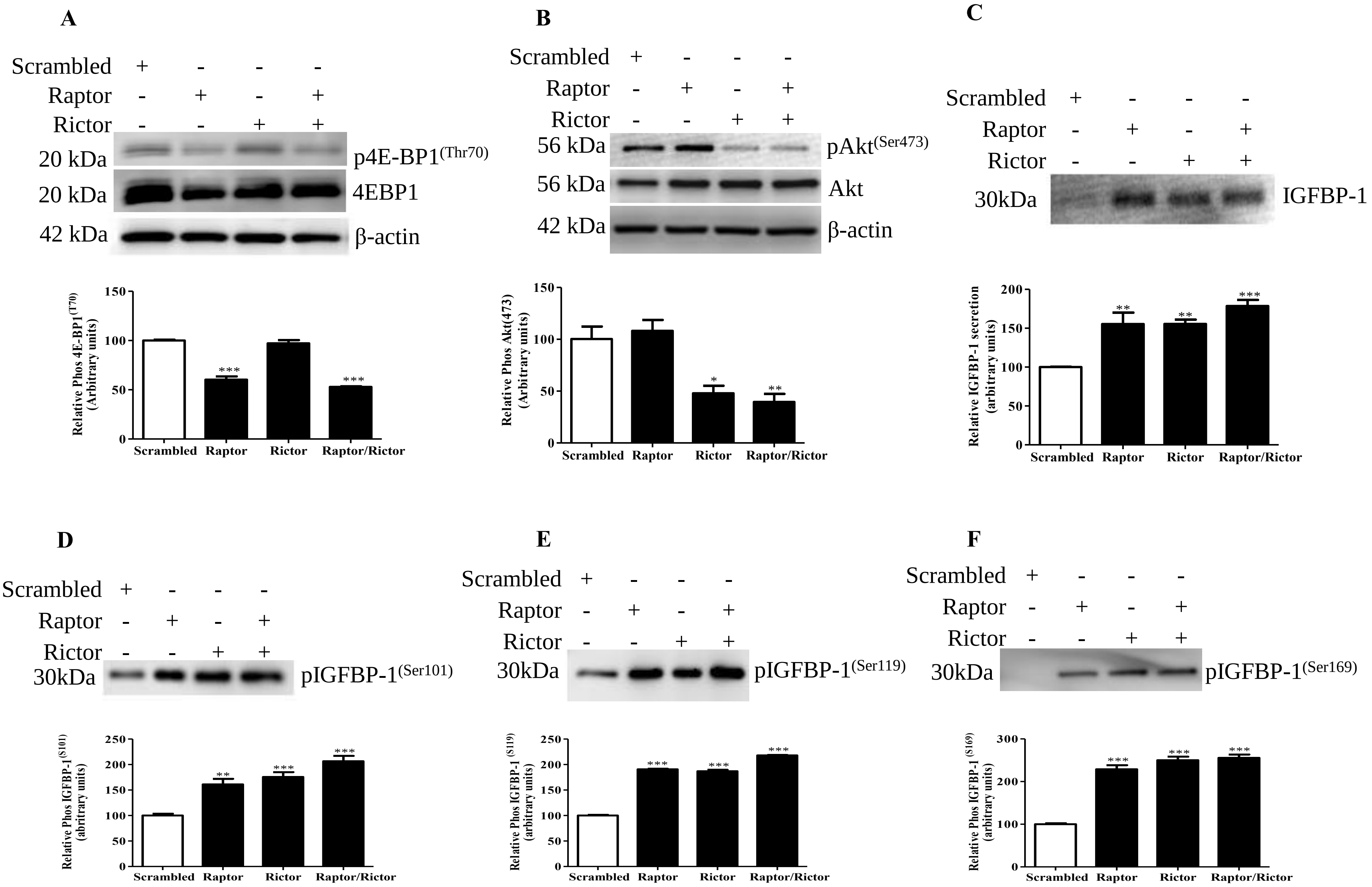

A
Scrambled + - - -
Raptor - + - +
Rictor - - + +
20 kDa p4E-BP1(Thr70)
20 kDa 4EBP1
42 kDa β-actin
Relative Phos 4E-BP1(T70) (Arbitrary units)
150
100
50
0
***
***
Scrambled
Raptor
Rictor
Raptor/Rictor
B
Scrambled + - - -
Raptor - + - +
Rictor - - + +
56 kDa pAkt(Ser473)
56 kDa Akt
42 kDa β-actin
Relative Phos Akt(473) (Arbitrary units)
150
100
50
0
*
**
Scrambled
Raptor
Rictor
Raptor/Rictor
C
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa IGFBP-1
Relative IGFBP-1 secretion (arbitrary units)
200
150
100
50
0
**
**
***
Scrambled
Raptor
Rictor
Raptor/Rictor
D
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser101)
Relative Phos IGFBP-1(S101) (arbitrary units)
250
200
150
100
50
0
**
***
***
Scrambled
Raptor
Rictor
Raptor/Rictor
E
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser119)
Relative Phos IGFBP-1(S119) (arbitrary units)
250
200
150
100
50
0
***
***
***
Scrambled
Raptor
Rictor
Raptor/Rictor
F
Scrambled + - - -
Raptor - + - +
Rictor - - + +
30kDa pIGFBP-1(Ser169)
Relative Phos IGFBP-1(S169) (arbitrary units)
300
200
100
0
***
***
***
Scrambled
Raptor
Rictor
Raptor/Rictor

Figure S1 (A-F)

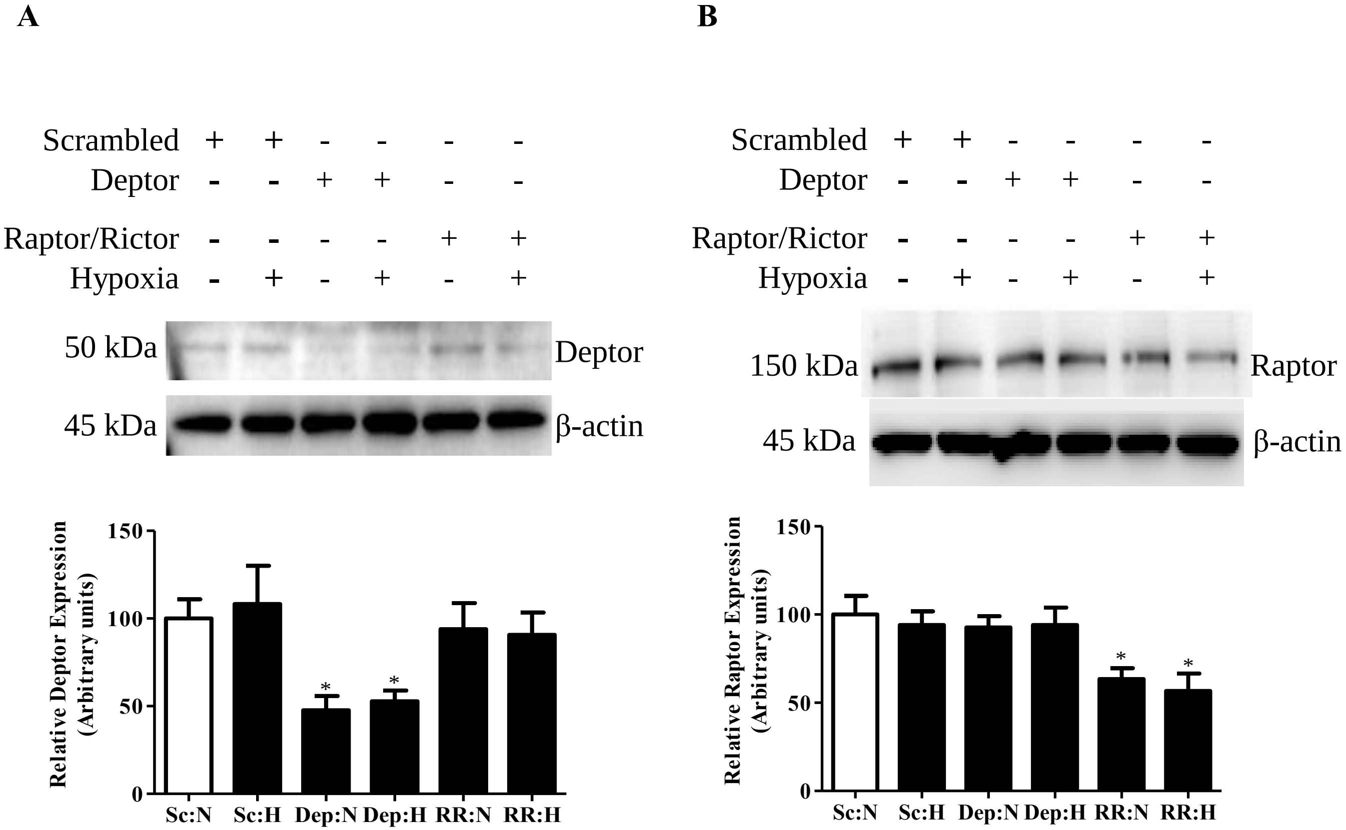

A
B
Scrambled + + - - - -
Deptor - - + + - -
Raptor/Rictor - - - - + +
Hypoxia - + - + - +
50 kDa
Deptor
45 kDa
β-actin
150 kDa
Raptor
45 kDa
β-actin
Relative Deptor Expression (Arbitrary units)
Relative Raptor Expression (Arbitrary units)
150
100
50
0
*
Sc:N
Sc:H
Dep:N
Dep:H
RR:N
RR:H

Figure S2 (A-B)

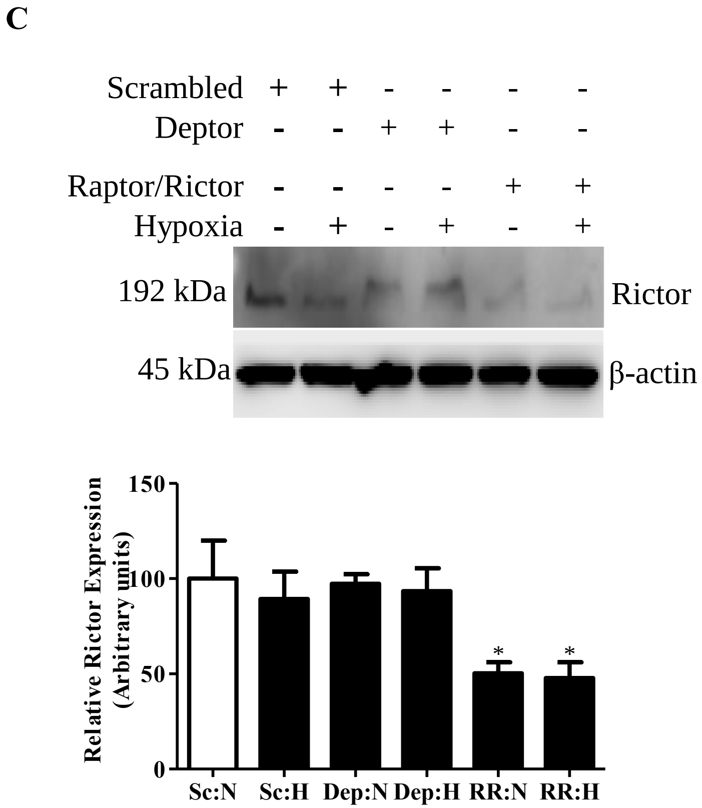

C
Scrambled + + - - - -
Deptor - - + + - -
Raptor/Rictor - - - - + +
Hypoxia - + - + - +
192 kDa
Rictor
45 kDa
β-actin
Relative Rictor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
Dep:N
Dep:H
RR:N
RR:H

Figure S2 (C)

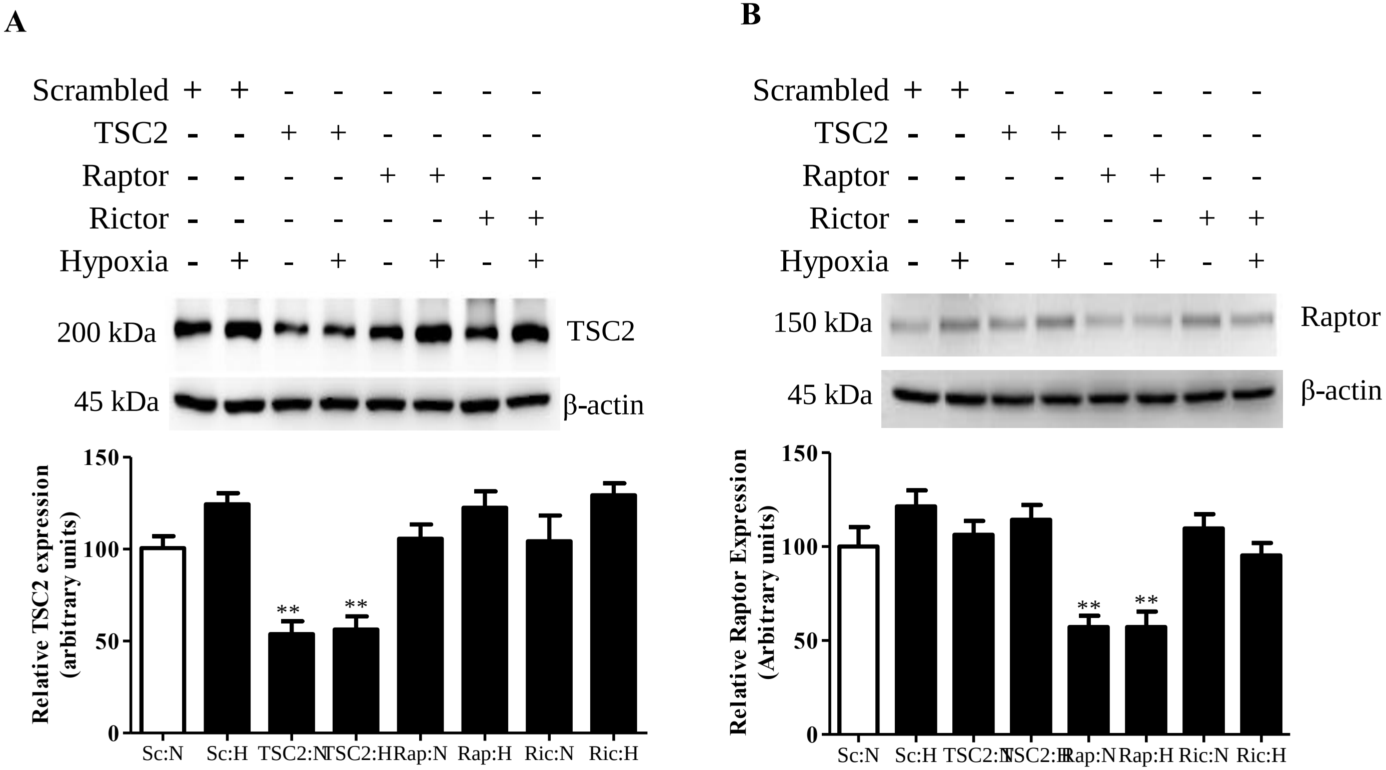

A
Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
200 kDa
TSC2
45 kDa
β-actin
Relative TSC2 expression (arbitrary units)
150
100
50
0
**
**
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H
B
Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
150 kDa
Raptor
45 kDa
β-actin
Relative Raptor Expression (Arbitrary units)
150
100
50
0
**
**
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H

Figure S3 (A-B)

C

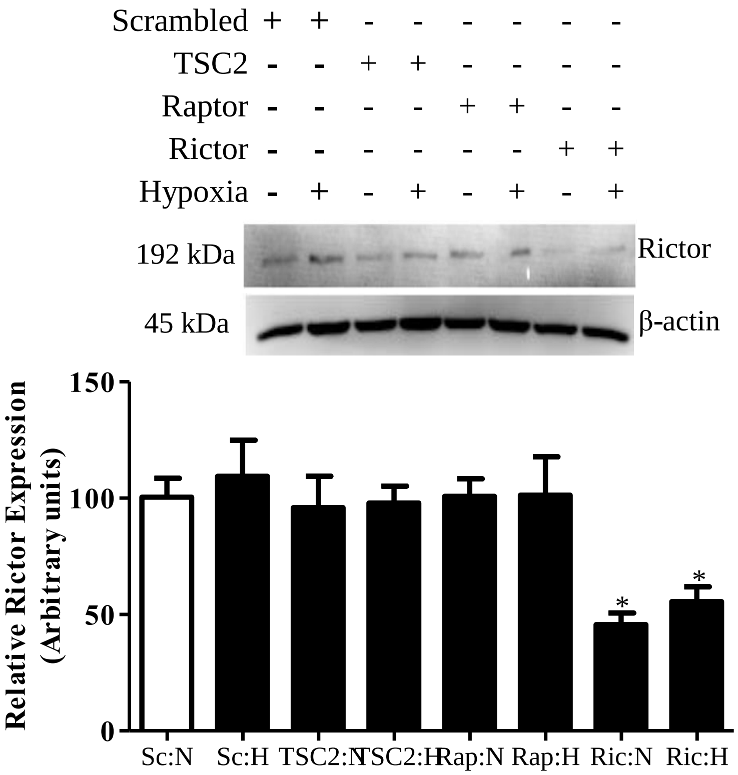

Scrambled + + - - - - - -
TSC2 - - + + - - - -
Raptor - - - - + + - -
Rictor - - - - - - + +
Hypoxia - + - + - + - +
192 kDa
Rictor
45 kDa
β-actin
Relative Rictor Expression (Arbitrary units)
150
100
50
0
*
*
Sc:N
Sc:H
TSC2:N
TSC2:H
Rap:N
Rap:H
Ric:N
Ric:H

Figure S3 (C)

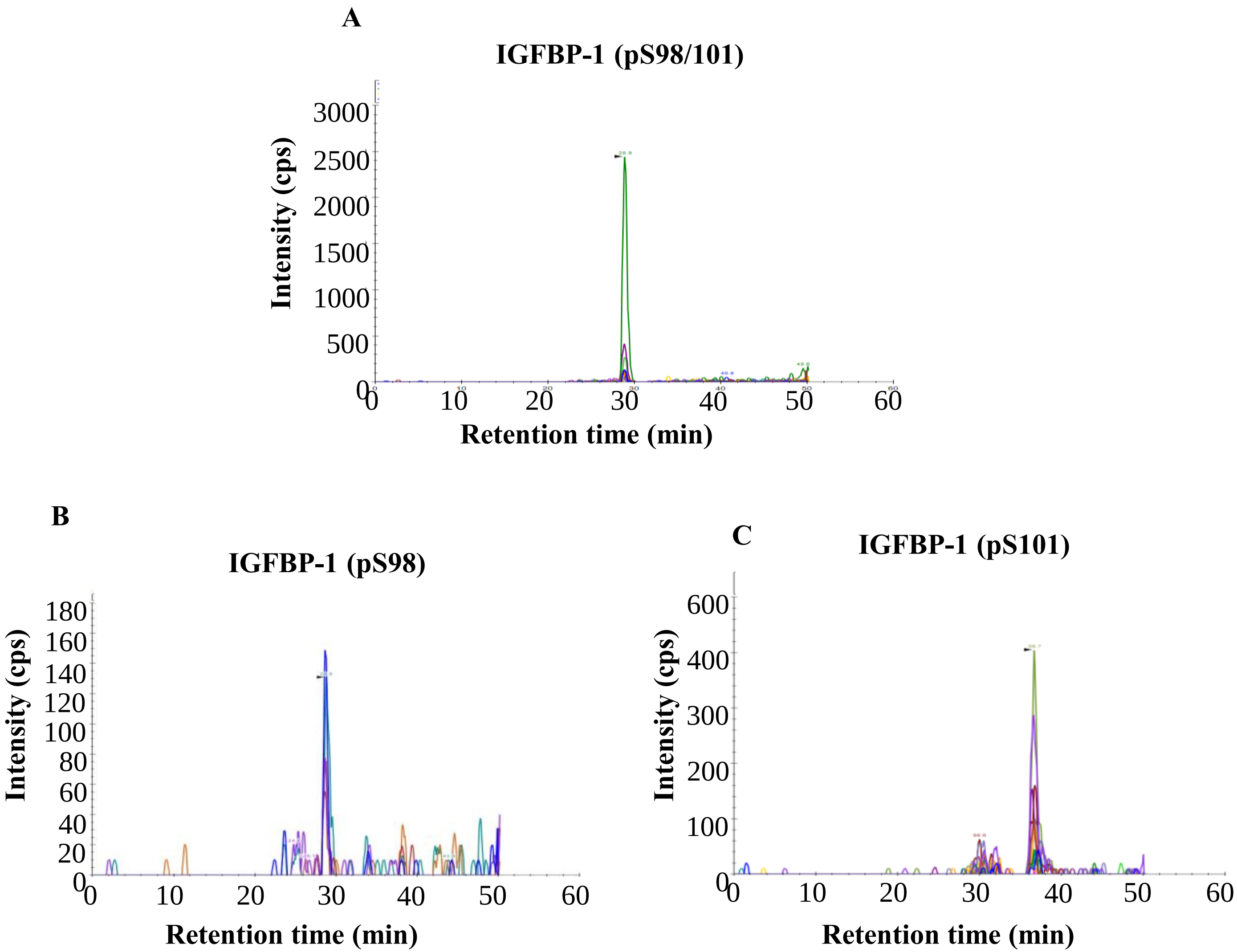

A
IGFBP-1 (pS98/101)
Intensity (cps)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Retention time (min)
B
IGFBP-1 (pS98)
Intensity (cps)
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Retention time (min)
C
IGFBP-1 (pS101)
Intensity (cps)
600
400
300
200
100
0
0
10
20
30
40
50
60
Retention time (min)

Figure S4 (A-C)

D

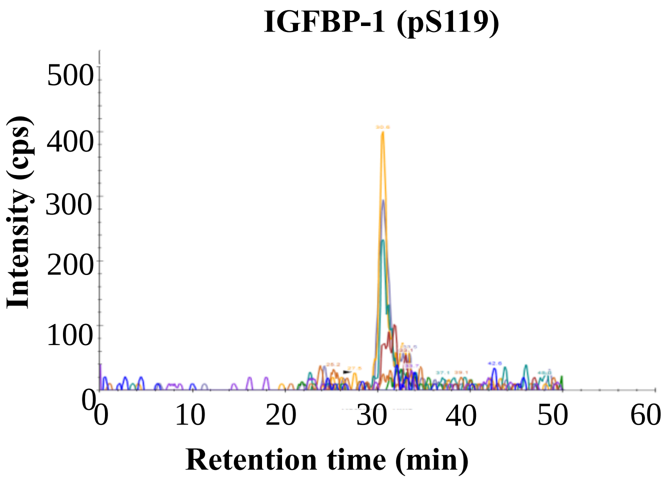

IGFBP-1 (pS119)
Intensity (cps)
500
400
300
200
100
0
0
10
20
30
40
50
60
Retention time (min)

Figure S4 (D)

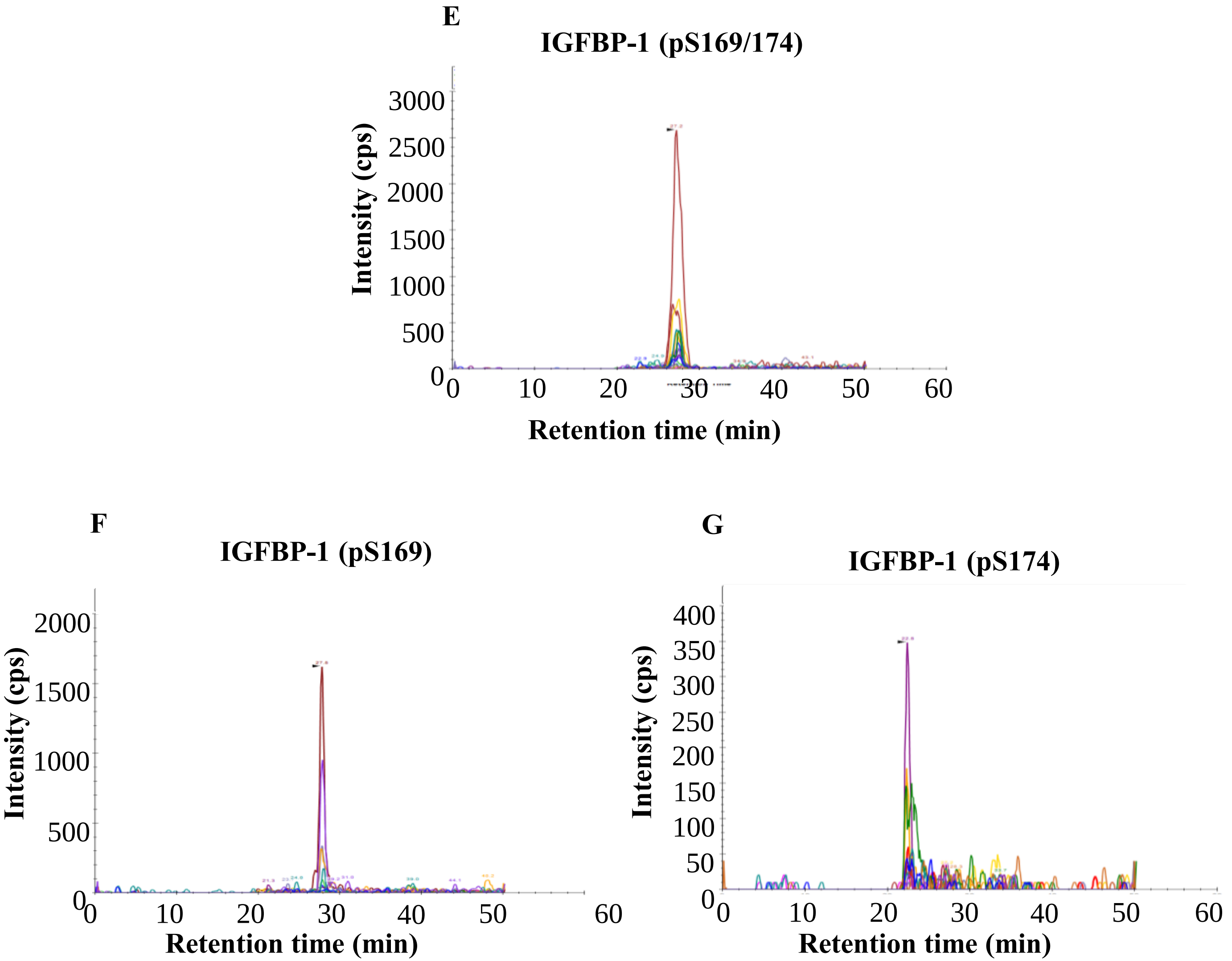

E
IGFBP-1 (pS169/174)
Intensity (cps)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
Retention time (min)
F
IGFBP-1 (pS169)
Intensity (cps)
2000
1500
1000
500
0
0
10
20
30
40
50
60
Retention time (min)
G
IGFBP-1 (pS174)
Intensity (cps)
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
Retention time (min)

Figure S4 (E-G)

H **Standard IGFBP-1 internal peptide**

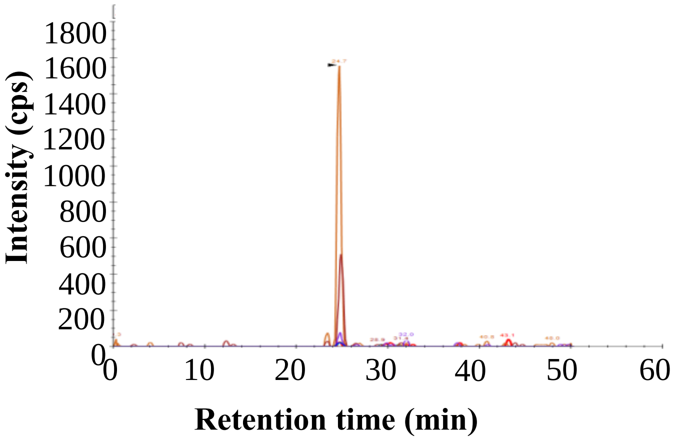
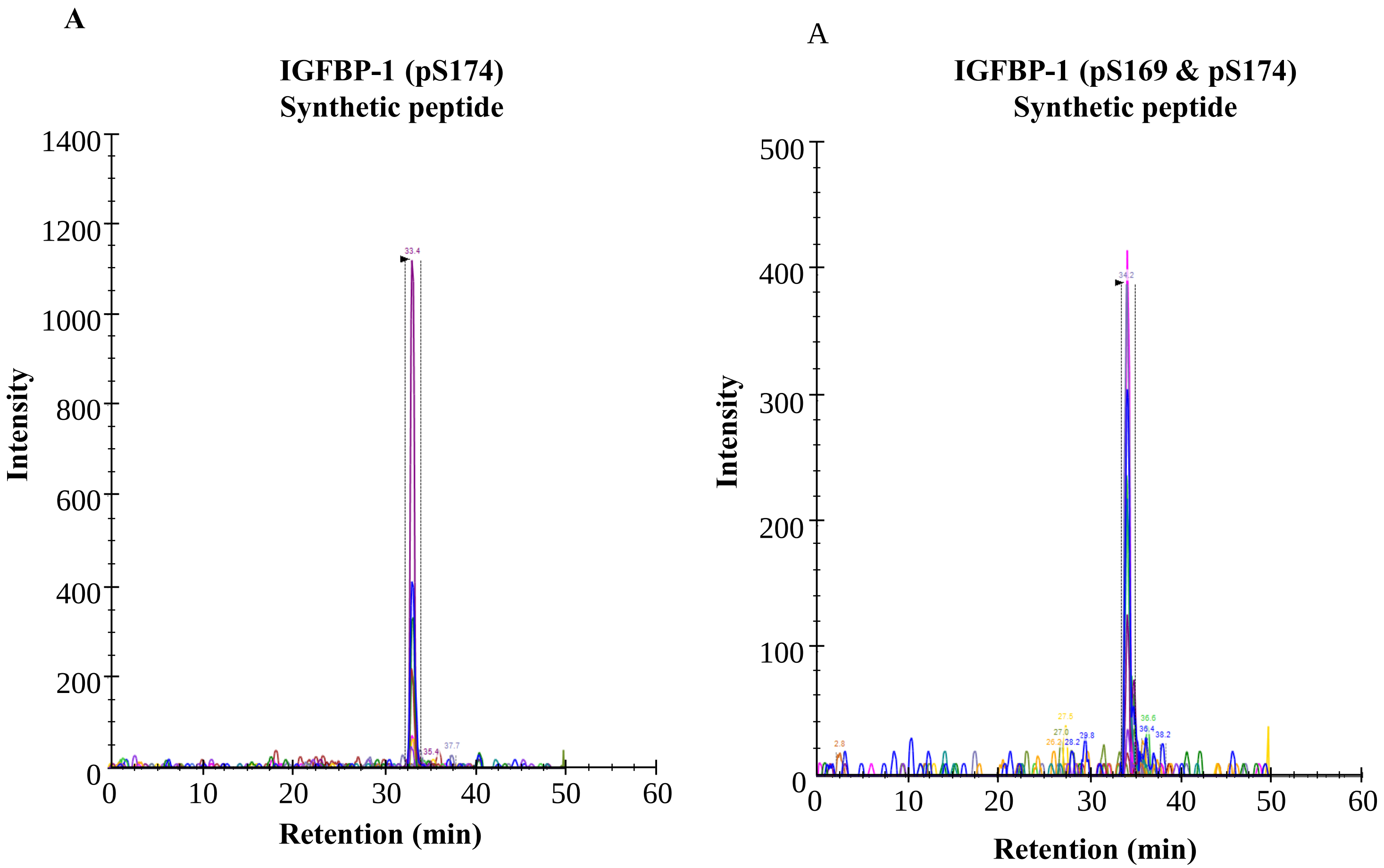


Figure S4 (H)


A

IGFBP-1 (pS174)
Synthetic peptide

A

IGFBP-1 (pS169 & pS174)
Synthetic peptide

Figure S5 (A-B)

Supplemental Figure 1. The effect of raptor and rictor silencing on IGFBP-1 secretion and phosphorylation

Representative western blot of A. mTORC1 readout 4E-BP1 (Thr70) and B. mTORC2 readout Akt (Ser473) phosphorylation in HepG2 cell lysates. Raptor and raptor+rictor silencing inhibited 4E-BP1 phosphorylation while rictor and raptor+rictor silencing both inhibited Akt phosphorylation to similar degrees. Western blot using equal aliquots of cell media of scrambled, raptor, rictor, and combined raptor+rictor siRNA treated HepG2 cells. Raptor, rictor, and combined raptor+rictor siRNA all significantly increased. **C.** IGFBP-1 secretion and **D-F.** IGFBP-1 phosphorylation at Ser101, Ser119 and Ser169, suggesting that inhibition of either complex is sufficient to induce IGFBP-1 secretion and phosphorylation. Values are displayed as mean ± SEM. $*p < 0.05$, $**p = 0.001\text{-}0.05$, $***p < 0.0001$;

Supplemental Figure 2. Validation of DEPTOR, raptor, and rictor siRNA on respective protein expression levels under hypoxia

Representative western blots of equal concentration of cell lysate protein from HepG2 cells transfected with scramble (normoxia), scramble (hypoxia), DEPTOR (normoxia), DEPTOR (hypoxia), raptor+rictor (normoxia) and raptor+rictor (hypoxia) siRNA. siRNA silencing of **A.** DEPTOR, B. Raptor and C. Rictor. DEPTOR significantly decreased total DEPTOR protein expression, regardless of hypoxic status. DEPTOR silencing did not affect either raptor or rictor protein expression. Raptor siRNA silencing decreased only raptor protein expression regardless of hypoxic status. Similarly rictor siRNA silencing specifically decreased rictor protein expression regardless of hypoxic status. Values are displayed as mean± SEM. $*p < 0.05$, $**p =$

0.001-0.05, ***$p <$ 0.0001. versus control; One-way analysis of variance; Dunnet's Multiple Comparison Test.

Supplemental Figure 3. Validation of TSC2, raptor, and rictor siRNA on respective protein expression levels under hypoxia

Representative western blots of equal cell lysate protein from HepG2 cells transfected with scramble (normoxia), scramble (hypoxia), TSC2 (normoxia), TSC2 (hypoxia), raptor (normoxia), raptor (hypoxia), rictor (normoxia) and rictor (hypoxia) siRNA. siRNA silencing of **A**. TSC2, **B**. Raptor and **C**. Rictor. TSC2 significantly decreased total TSC2 protein expression regardless of hypoxic status. Raptor siRNA significantly decreased specifically total raptor protein expression, regardless of hypoxic status. Rictor siRNA significantly decreased only rictor protein expression, regardless of hypoxic status. Values are displayed as mean ± SEM. *$p< 0.05$, **$p=$ 0.001-0.05, ***$p <$ 0.0001. versus control; One-way analysis of variance; Dunnet's Multiple Comparison Test.

Supplemental Figure 4. Representative MRM spectra of identified phosphorylation sites

Immunoprecipitated IGFBP-1 samples or a standard synthetic IGFBP-1 internal peptide was digested by respective enzymes to generate appropriate peptide fragments. MRM transitions were predicted by Skyline and the transitions **A-H** representing the modification sites on IGFBP-1 singly and in combination were chosen to distinguish from other serine residues.

Supplemental Figure 5. MRM spectra of synthetic IGFBP-1 peptides containing the novel Ser174 phosphorylation modification

Synthetic peptides of the digested fragment of IGFBP-1 containing either **A**. pSer174 or **B**. pSer169+pSer174 phosphorylation. Synthetic peptides were used to validate the transitions used to detect the novel pSer174 phosphorylation site within IGFBP-1.